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Extracellular Enzyme Activities of Storage Fungi Isolated from Some Medicinal Plants of Maharashtra

Jadhav Ramesh Rangrao

Department of Botany, M.B.S.K. KanyaMahavidyalaya,Kadegaon, Dist.-Sangli – 415304 (M.S.) India.

ABSTRACT: Medicinal plants are largely cultivated in Maharashtra. They are found to be heavily infected with variety of fungi in field and storage. These associated fungi are known to deteriorate the plant parts and its chemical contents. These associated fungi isolated from the different plant parts during storage in gunny bags in store houses.Isolated fungi were screened qualitatively and quantitatively for production of extracellular enzyme amylase, Effect of different carbohydrates on amylase production have been studied and found that glucose, fructose and lactose when supplemented in the basal medium to be stimulatory.Regarding the effect of supplementation of carbohydrates on amylase production in six fungi showed variation in their selection for carbohydrates to stimulate amylase production.

KEY WORDS: Amylase production, storage fungi, medicinal plants

I. INTRODUCTION

Medicinal plants are extensively cultivated throughout India.The plant parts after harvesting they stored in store houses or godowns in gunny bags.They are found to be heavily infested with variety of fungi, these associated fungi are known to deteriorate the plant parts and its contents. The isolation and identification of fungal pathogens on different medicinal plants and their plant parts. For this isolation of fungi was made from medicinal plants collected from different regions of Maharashtra at different age and different varieties of medicinal plants in field and in storage conditions. It was interesting to observe that, different medicinal plants and their plant parts specially *Withania somnifera, Rauwolfia serpentina, Glycyrrhiza glabra, Emblica officinalis, Asparagus racemosus, Chlorophytum borivilianum, Zingiber officinale* showed association of different fungi having different physiological behavior. Biodeterioration of raw materials of six medicinal plants was examined,some of the contaminated raw materials were found to be deteriorated by toxigenic strains of Aspergillus flavus (Ashok Kumar et al 2009). During studies on survey of medicinal plant diseases it was interesting to note that following diseases were highly destructive to the crops in live field and storage conditions. Therefore six pathogenic moulds like *Aspergillus flavus, Curvularia lunata, Alternaria alternata, Fusarium oxysporum, Phytopthora sp., Rhizoctonia solani* were screened for their ability to produce amylase enzyme.

Regarding the effect of supplementation of carbohydrates on amylase production, the fungi showed variation in their selection for carbohydrates to stimulate amylase production. Role of extracellular enzyme amylase produced by stored drug plant parts fungi, during the process of deterioration of plants parts has been considered to be important ability to the fungi.Fazilath Uzma et al (2016) showed that endophytic fungal diversity and extracellular enzyme activity from the endangered plants. The fungal isolates were screened for the production of extracellular enzymes, of

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which 29% was positive for amylase, The fungal isolates were subjected for extracellular enzyme production, 29% of the isolates hydrolyzed starch and were positive for amylase activity The role of such enzyme amylasein case of seed pathogens have been studied by few workers, as Vidhasekaran et al. (1966) claimed that the production of extracellular hydrolytic enzymes by *Fusarium moniliforme* and *Aspergillus flavus* was found to be responsible in the spoilage of paddy seeds.During the process of biodeterioration extracellular hydrolytic enzymes plays very important role in the invasion and establishment of plant pathogen (Bateson and Miller,1966, Wood,1967). Regarding medicinal plant parts fungi veryscanty information is available about their role in biodeterioration of medicinal plant parts.

Preliminary studies on amylase production by various types of micro-organism in-vitro have been done by several workers as the species of *Penicillium and Aspergillus* (Mense et al.,1947),*Alternaria tenuis , Fusarium coeruleum and Curvularia lunata* (Tondon, 1949). Das et al. (1961) reported amylase production in some pathogenic fungi. *Aspergillus* and *Fusarium* have been reported to produce amylase in various media (Chahal et al. 1972). Production of amylase by fungi was found to be increased with the increase in the concentration of starch in the medium (Chapman et al. 1975). Effect of different carbohydrates on amylase production have been studied by Admas and Deploey (1976) and found that glucose, fructose and lactose when supplemented in the basal medium to be stimulatory.Wadje and Deshpande (1977) studied amylase production in seed mycoflora of jawar and found that the jawar meal medium superior than the starch medium. Fashim et al. (1985) noted that *Aspergillus flavus* as more efficient amylase producer than *A. niger*. Stimulatory effect of different carbohydrates on amylase production have been reported in case of *A. niger* due to maltose (Barton et al., 1972), due to dextrin in case of A. awamorii (Musaeva, 1967) and glucose along with starch in case of *A. flavus* (Khairnar, 1987).On the other hand, glucose alone proved inhibitory for amylase production in *Aspergillus oryzae* (Fenikrova et al., 1965), *A. fumigatus* and A.*terreus* (Venkateswarlu and Reddy, 1987).

II. MATERIAL AND METHODS

Amylase Enzymes of Fungal Pathogens:

a) Production :

Production of amylase was studied by growing the fungi on liquid medium containing starch 1% , KNO3 0.25%, KH_2PO_4 0.1% and MgSO4 7H2O 0.05%, pH of the medium was adjusted to 5.5.Twenty five ml of the medium was poured in 100 ml Erlenmeyer flasks and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 1 ml spore/mycelial suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at 25 ± 1^0C with diurnal periodicity of light. On 7^{th} day, the flasks were harvested by filtering the contents through WhatmanNo.1 filter paper. The filtrates were collected in pre-sterilized bottles and termed as crude enzyme preparations.

b) Enzyme assay (cup – plate method) :

Determination of amylase activity was done with the help of cup-plate method which was adopted by Singh and Saksena (1982), Where 25 ml of starch agar assay medium (soluble starch – 10 gm, Na_2PO4- 2.84 gm, Nacl – 0.35 gm, agar 20 gm, D.W. – 1000 ml and at pH 6.9). 15 ml of the medium were poured in each petriplate. On solidifying the medium, a cavity (8 mm diameter) was made in the center with the help of a cork borer (No.4) and was filled with 1 ml culture filtrate(Crude enzyme preparation). The plate were incubated at 28 ^{0}C for 24 hours then they were flooded with Lugol's iodine solution as an indicator. A clear, non blue, circular zone was obtained surrounding the central cavity. The diameter, which was measured (mm) as the amylase activity zone. Similar procedure was followed for the control except pouring of autoclaved culture filtrate in the central cavity instead of the active enzyme.

c) Composition of media used for amylase production :

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The synthetic media were employed for the production of amyalse in the preliminary experiment. Composition of media is given below.

1) **Starch nitrate medium :**

Soluble starch	-	10 gm
KNO_3	-	2.5 gm
KH_2PO_4	-	1.o gm
$MgSO_4.7H_2O$	-	0.5 gm

Dissolved in 100 ml of D.W.

2) **Glucose nitrate medium :**

Glucose	-	10 gm
KNO_3	-	2.5 gm
KH_2PO_4	-	1.0 gm
$MgSO_{4.7H2O}$	-	1.0 gm

Dissolved in 1000 ml of D.W.

III. RESULTS & DISCUSSION

Table :01 Effect of carbohydrates on amylase production in medicinal plants fungi:

Carbohydrates (0.5 % conc.)	Fungi					
	Alternaria alternata	*Aspergillus flavus*	*Curvularia lunata*	*Fusarium oxysporum*	*Phytopthora sp.*	*Rhizoctonia solani*
	Activity zone (mm)					
Monosaccharides						
Glucose	20	23	24	19	16	20
Fructose	19	20	22	16	15	21
Galactose	17	18	18	16	18	18
Mannose	16	17	19	18	16	16
Xylose	19	20	19	17	16	22
Disaccharides						
Sucrose	21	23	20	20	18	22
Maltose	12	16	14	10	16	17
Lactose	20	18	22	12	15	18
Polysaccharides						
CMC	16	15	14	13	14	12
Pectin	17	20	18	10	12	16
Mannitol	20	22	20	18	19	20

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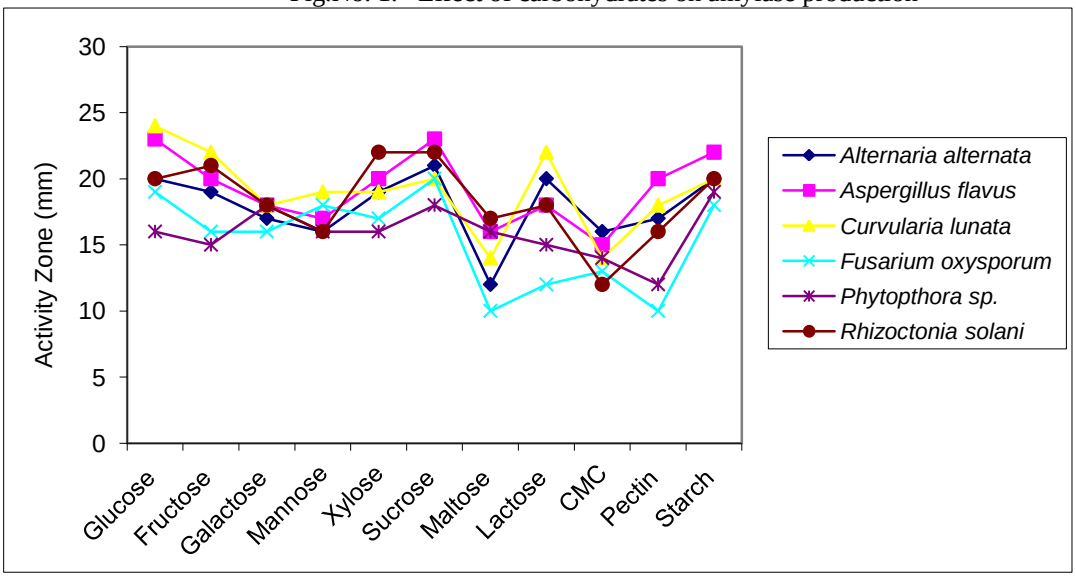
Among the eleven fungi isolated from different medicinal plants parts during storage, most of them produced amylase in the medium with or without substrate. This clearly indicates that amylolytic nature was found to be common character in all the fungi which are associated with drug plant parts. Similarly amylase production in these fungi in absence of starch indicated constitutive nature of amylase production. This clearly suggest that addition of starch (substrate) was found to be favourable to accelerate the process of amylase production *Aspergillus flavus, A. niger,Fusarium oxysporum,Curvularia lunata,Alternaria alternata* and *Aspergillus terreus* were found to be highly amylolytic in nature. This gives an idea that the amylolytic nature was directly related with the dominance of fungi. Such type of constitutive nature of amylase production has been reported in species of *Alternaria, Curvularia* and *Helmintho sporium* (Khairnar, 1987), *Aspergillus flavus,Aspergillus niger,Curvularia lunata,Fusarium oxysporum, F. moniliforme* (Bhosale, 1989).

Regarding the effect of supplementation of carbohydrates on amylase production, in was seen (Table 01) that, the fungi showed variation in their selection for carbohydrates to stimulate amylase production. This indicates the specificity of carbohydrates during synthesis of the fungal amylase. Among different carbohydrates, sucrose proved stimulatory for amylase production in all the six fungi, Maltose proved inhibitory for *Fusarium oxysporum,* CMC proved poor source of carbon for all the six fungi, pectin and mannitol for *Aspergillus flavus* and *Curvularia lunata,* proved stimulatory for amylase producton indicating that, these fungi have different choices for carbohydrates during increased metabolic activities like production of enzymes. The effect of carbohydrates was studied by different workers maltose in case of *A. niger* (Barton et al, 1972), glucose along with starch in case of *A. flavus* (Khairnar, 1987), glucose, maltose and starch in case of *Phomaexigua* and *Graphium penicillioides*(Charya and Reddy, 1980), were found to be stimulatory for amylase production.

Fig.No. 1. Effect of carbohydrates on amylase production



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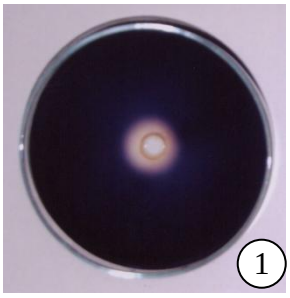
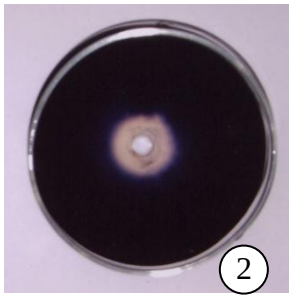
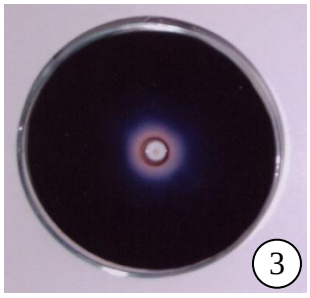
AMYLASE ZONE OF ACTIVITY IN DIFFERENT FUNGI



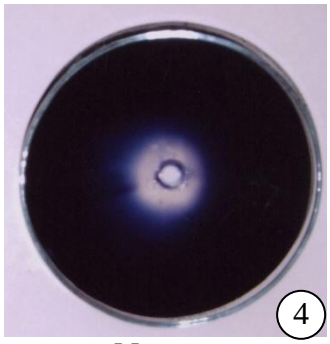
Glucose



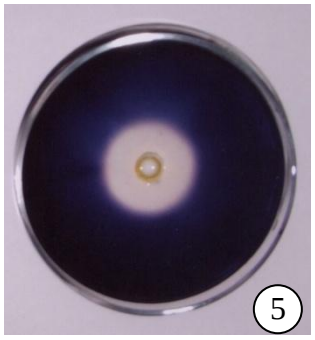
Fructose



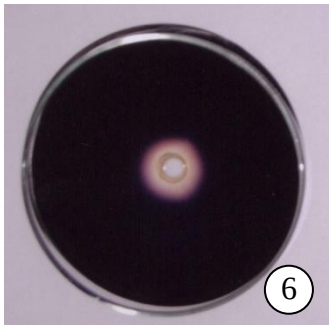
Galactose



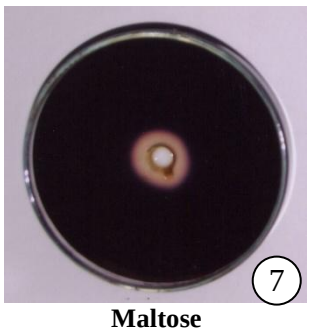
Mannose



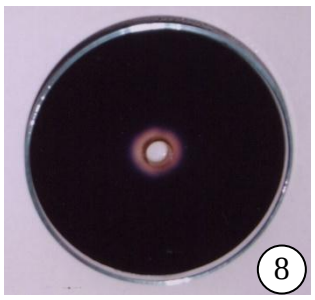
Xylose



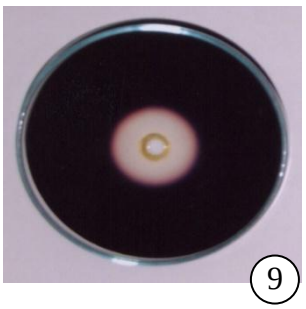
Sucrose



Maltose



Lactose



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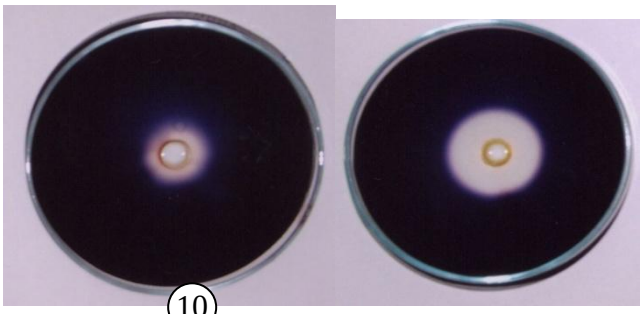

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IV.CONCLUSION

1) Among the total eleven fungi isolated from drug plants were capable to produce both amylase enzymes.
2) All the eleven medicinal plant parts fungi produce amylaseon both substrate and non substrate media.
3) The degree of amylase production increased in the presence of starch (substrate) medium than in glucose nitrate (Non-substrate) in all the fungi.
4) Among carbohydrates, sucrose is proved to be stimulatory as compared with other carbohydrate sources which indicate that fungi have different choice of carbohydrates among fungi.

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